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MULTILYTE LTD.

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

SAN FRANCISCO DIVISION

AFFYMETRIX, INC., a Delaware corporation,

Plaintiff and Counterdefendant,

v.

MULTILYTE LTD., a British corporation,

Defendant and Counterclaimant.

Case No.: C-03-3779 WHA

REPLY DECLARATION OF DR. LARRY J. KRICKA IN SUPPORT OF MULTILYTE LTD.'S CLAIM CONSTRUCTION REPLY BRIEF

I, Larry J. Kricka, declare:

1. I submit this declaration in support of Multilyte Ltd.'s Claim Construction Reply Brief. In particular, I have been asked to respond to certain points raised in Affymetrix, Inc.'s Responsive Claim Construction Brief and the declaration of Edwin F. Ullman submitted in support of that brief.

Specificity of Nucleic Acid and Antibody Binding

2. In order for a binding assay to perform its intended function, the binding agent used in the assay must be sufficiently specific for the analyte. This means, in general, that the binding agent must bind the analyte to a greater degree than other substances in the sample that are not of interest in the assay. This specificity requirement exists for all binding assays, regardless of whether the binding agent is an antibody, a nucleic acid, or some other substance.

3. In the case of DNA microarrays, which are binding assays that use oligonucleotides as the binding agents, the binding agents must be sufficiently specific for the single-stranded nucleic acids in the sample that are being detected or measured. If a particular oligonucleotide binding agent were not specific for an analyte—that is, if it bound many substances in the sample to more or less the same degree—the user of the assay would not be able to derive any useful information from the use of that binding agent. This is because the user would not know if the binding agent was binding to the substance of interest or to some other substance present in the sample. Accordingly, DNA microarrays rely on the specific binding interactions between single-stranded nucleic acids in order to provide useful results.

4. Specificity of binding is a matter of degree, and some interactions are more specific than others. This is true both for nucleic acid binding and for antibody-antigen binding. The binding of a substance other than the analyte to the binding agent is referred to as a "cross-reaction," and it can interfere with the accuracy of the assay. Cross-reactivity can present problems for nucleic acid binding assays and for immunoassays (binding assays using antibodies). One of ordinary skill in the art of binding assays would understand the importance of selecting a binding agent for a particular binding assay that will not cross-react in a way that will detract from the accuracy of the assay.

5. The ranges of cross-reactivities experienced with antibody binding can be seen from the commercially available immunoassay kits that are currently on the market. Attached hereto as Exhibits A, B, and C are three package inserts for three different commercially available binding assays that rely on antibodies as the binding agents. The first of these is an assay designed to detect the presence of HCG, a hormone that is monitored for the early detection of pregnancy. This assay is highly specific, as indicated by the fact that the package insert states that the cross-reactivity with three other hormones that are likely to be present in the sample along with the HCG (that is, luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH)) were shown to be 0.09%, 0.2%, and 0.02%, respectively. *See* Ex. A at 8 (under "Specificity"). These are very low rates of cross-reactivity.

6. On the other hand, Exhibit B is a package insert for an assay that uses an antibody to detect amphetamines. The antibody used in this assay engages in non-specific binding, as indicated by the fact that the antibody binds to several different substances more or less equally. *See* Ex. B at 3, Table 7. The reason that this cross-reactivity does not present problems for this particular assay is that all of the different substances that bind to the antibody are amphetamine compounds. Therefore, the presence of any of these substances indicates the presence of an amphetamine, meaning that, in some sense, the user of the assay is indifferent to which of the substances is being detected. Nonetheless, this assay presents an example of antibody binding that is highly non-specific.

7. Another example of non-specific antibody binding is shown in Exhibit C, which is a package insert for a binding assay used to detect the presence of opiates. Again, the antibody used in this assay binds non-specifically to several different opiate compounds. *See* Ex. C at 4, Table 7. This package insert also notes, however, that:

> Therapeutic doses of ofloxacin (Floxin) or levofloxacin (Levaquin), *non-opiates*, may produce positive results with this assay. A positive result from an individual taking ofloxacin or levofloxacin should be interpreted with caution and confirmed by another method.

Id. at 4 (text above Table 7) (emphasis added). This means that the antibody used in this binding assay is non-specific to the point that it cross-reacts with substances that are not opiates, which,

depending on whether the subject of the test has been taking certain medicines, could significantly interfere with the accuracy of the test.

The Use of the Term "Binding Agent"

8. The exact term "binding agent" has been used many times to refer to nucleic acids. Attached hereto as Exhibits D through H are copies of five U.S. patents, issued between 1976 and 1989, all of which use the term "binding agent" in this manner. *See* Ex. D (U.S. Patent No. 4,000,252) at col. 3, line 64 to col. 4, line 30; Ex. E (U.S. Patent No. 4,719,176) at col. 20, lines 47-49; Ex. F (U.S. Patent No. 4,868,130) at col. 18, lines 51-53, col. 20, lines 48-58; Ex. G (U.S. Patent No. 4,886,761) at col. 2, line 50 to col. 3, line 18; Ex. H (U.S. Patent No. 4,769,121) at col. 1, lines 47-52, col. 4, lines 40-42, col. 5, lines 19-35.

The Magnitude of Affinity Constants for Nucleic Acids

9. I understand that Affymetrix has taken the position that the affinity constants applicable to the hybridization of nucleic acids are significantly higher than 10^{13} liters/mole. This has prompted me to research the scientific literature to find the values of affinity constants that have been experimentally determined for nucleic acid binding. All of the literature that I have found on the topic shows that affinity constants for the binding of single-stranded nucleic acids are less than 10^{13} liters/mole.

10. Attached hereto as Exhibit I is a 1973 paper that reports an affinity constant (referred to as the "association constant" in the paper) for the binding of single-stranded nucleic acids of 8.6 x 10^6 liters/mole. *See* Ex. I at 809. 10^6 is 10,000,000 times less than 10^{13}.

11. Attached hereto as Exhibit J is a 1970 paper that reports an affinity constant (or "K" value) for the binding of single-stranded nucleic acids of 0.047 μg/mL. *See* Ex. J at 227. This value can be converted into liters/mole by using the molecular weight of 1.5 x 10^6 grams/mole that is provided in the paper. *See id.* Once this calculation is performed, the "K" value is determined to be approximately 3 x 10^{10} liters/mole, which is about 1000 times less than 10^{13}.

12. Attached hereto as Exhibit K is a 1968 paper that reports a "disassociation constant" for the binding of single-stranded nucleic acids of about 10^{-10} moles/liter. *See* Ex. K at 276. A disassociation constant of 10^{-10} moles/liter translates into an affinity constant of 10^{10} liters/mole,

which is about 1000 times less than 10^{13}.

13. Attached hereto as Exhibit L is a 2001 paper that reports an affinity constant for the binding of single-stranded nucleic acids to oligonucleotides that are 18 nucleotides long and that are attached to a solid support in a microarray format. The paper reports an affinity constant (referred to as the "adsorption coefficient" in the paper) of 1.8×10^7 liters/mole. 10^7 is 1,000,000 times less than 10^{13}.

I declare under penalty of perjury pursuant to the laws of the United States that the foregoing is true and correct. Executed this 29th day of April, 2004 in San Jose, California.

Larry J. Kricka